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Histopathological examination of gunshot wounds

Forensic pathology of gunshot wounds

Ali Tavasli[1], Erdem Okdemir[2], Safa Celik[1], Muzaffer Yildirim[1], Muhammet Demir[1], Alper Keten[3], Mehmet Siddik Tuncay[4]
1 Department of Forensic Medicine, Branch Office, Gaziantep, Turkey
[2] Department of Forensic Medicine, Gokyuzu OSGB Lmt., Gaziantep, Turkey
[3] Department of Forensic Medicine, Institute of Forensic and Traffic Medicine, Heidelberg University, Germany
[4] Department of Physical Medicine and Rehabilitation, Liv Hospital, Gaziantep, Turkey

Abstract

Aim: Deaths resulted from gunshot injuries have forensic characteristics. Autopsies are required in order to determine the number of shots, direction of shots, distance of shots, deadly wounds etc. Histopathologic examination on wounds is one of the methods that help investigations. In this study, we aimed to define histopathologic parameters of entrance-exit wounds in gunshot injuries. Moreover, we tried to determine usability of the defined parameters in entrance-exit wounds differentiation and estimation of shooting distance.

Material and Methods: 113 gunshot wounds of which 62 entrance and 51 exit wounds are investigated in our study. Necrosis on epidermis and dermis; presence of foreign body (black pigmented particles, gunpowder; textile fibers) on epidermis and dermis; bleeding on dermis and subcutaneous tissues; presence of fat, bone and muscle tissue on wound tract are evaluated histomorphologically.

Results: Skin necrosis, widespread dermal bleeding, textile fibers and gunpowder on wound tracts were statistically high on entrance wounds. Presence of fat, muscle and bone tissues on wound tracts and absence of skin necrosis, gunpowder particles and textile fibers were statistically high on exit wounds. It was observed that gunpowder particles and necrotic tissue between gunpowder decreases as shooting distance increases. It was found that attained data were aligned with the literature.

Discussion: Along with other methods, histopathologic assessments support the verification of diagnosis. Moreover, it is helpful in making decision on whether the wound is a gunshot injury or not; entrance-exit wound differentiation and estimation of shooting distance in cases of corpses without clothes and interfered corpses or wound characteristics could not be assessed macroscopically.

Keywords

Forensic Pathology, Gunshot Wound, Histology, Microscopy, Range Of Fire

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Corresponding Author: Ali Tavasli, Department of Forensic Medicine, Branch Office, Gaziantep, Turkey.
E-mail: alitavasli20@gmail.com P: +90 533 957 20 35
Corresponding Author ORCID ID: https://orcid.org/0000-0002-7661-5357
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Introduction

In gunshot wound cases, differentiating the entrance wounds from the exit wounds and estimating the range of fire are important for the reconstruction of crime scenes and determining the manner of death. Such evaluations are mainly performed by detecting the gunshot residues (GSR) macroscopically. In cases where there is no GSR, the range of fire is considered a distant shot. However, in cases involving medical interventions, decomposition, and burning, the detection of GSR becomes difficult [1, 2]. Various microscopic examination methods have therefore been conducted, and these have included hematoxylin and eosin (H&E) and sodium rhodizonate staining, and Fourier-transform infrared (FT-IR) microscopy [3, 4]. Dolinak et al pointed out that FT-IR microscopy could be of benefit in cases where macroscopic examination is difficult, such as medical intervention, decomposition, and burning [5]. Marty et al indicated that measuring the density and distribution of the staining reaction using sodium rhodizonate as a histological staining technique is an effective method to determine the range of fire and the entrance wound [6].

Perez and Molina's study of H&E showed that a bullet can carry powder grains to the exit wound [7]. Microscopic examination of gunshot wounds is therefore not recommended for routine assessments and there is thus little data on this subject [8, 9]. For this reason, the inclusion of a large volume of relevant data will help guide the evaluation of selected cases. In this study, we discuss the findings obtained using a standard microscopic evaluation of gunshot wounds with a relatively large sample group. A total of 113 wounds from 45 cases were examined macroscopically and microscopically.

Material and Methods

This study was approved by the Ethics Committee of Council of Forensic Medicine, Istanbul (Date: 27/01/2015, No: 2015/14). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. In this study, deaths determined to be the result of gunshot wounds by the Morgue Department of the Council of Forensic Medicine were evaluated prospectively. Wounds sustained via short-barrel guns were evaluated. The type of barrel used was determined based on the descriptions of the ammunition obtained from the autopsy and criminal laboratory reports. The time of death of the cases did not exceed 36 hours. A total of 113 wounds from 45 cases were examined macroscopically and microscopically. For entrance wounds in clothing areas, the range of fire was determined according to the findings of the crime scene and the criminal laboratory reports.

In cases where the bullet was not present in the body, descriptions of the bullet were obtained from the crime scene investigation reports. A scalpel was used to sample each lesion at a radius of about 3 cm and a depth of 2 cm from the center of the entrance or exit hole. The samples were fixed in 10% formaldehyde for at least 48 hours. Samples with a thickness of approximately 3 mm were taken from the fixed tissues in parallel with the bullet trajectory. After tissue tracking, the tissues were embedded in paraffin blocks. From the paraffin blocks, sections with a 3–5 μm thickness were taken using a microtome. The prepared samples were stained with H&E and evaluated under a light microscope.

For the entry and exit wounds, necrosis in the epidermis and dermis, foreign bodies in the epidermis and dermis (black-pigmented particle powder and textile fibers), bleeding in the dermis and subcutaneous tissue, and the presence of fat, muscle, and bone tissue in the wound tract were examined. In addition, the relationship between the range of fire and the diameter of the bullet was evaluated using the histomorphological parameters described above. The presence of necrosis in the epidermis was divided into two groups: mild and severe. If the partial necrosis of keratinocytes was limited in the wound edges, it was considered to be mild. Contrarily, if the necrosis was intense and covered large areas, it was evaluated as severe. Light eosinophilic changes in the collagen around the wound trauma in the dermis were considered mild dermal necrosis, and basophilic changes in the collagen were considered severe dermal necrosis. Severe necrotic connective tissue elements were evaluated as "compact" if they maintained integrity under the epidermis and were considered "fragmented" if they disintegrated in the wound path. Bleeding in the subcutaneous tissue and dermis were classified as "focal" if it was partial and "common" if it was broad.

Statistical Analysis

All the data were analyzed with SPSS version 14.0 (Statistical Package for the Social Sciences) software for Windows. Individual and aggregate data were summarized using descriptive statistics including mean, standart deviations and percentages. For initial evaluations and comparison of the data with normal distribution, the Kolmogorov-Smirnov test was used. A statistical analysis of the collected data was conducted via a chi-square test using SPSS version 21.0 (IBM Inc., NY/USA). The level of statistical significance was accepted as $p<0.05$.

Results

Among the 113 gunshot wounds, 62 (54.9%) were entrance wounds, and 51 (45.1%) were exit wounds. Of the 53 wounds for which the range of fire was evaluated by macroscopic examination, 18 (34%) were contact wounds, 9 (17%) were close-range wounds, and 26 (49%) were distant-range wounds. Of the 59 bullets that created entrance wounds, 1 (1.7%) was 6.35 mm in diameter, 23 (39%) were 7.65 mm in diameter, and 35 (59.3%) were 9 mm in diameter.

The relationship between epidermal/dermal necrosis and the entrance/exit wounds was examined (Figure 1).

While the incidence of severe epidermal/dermal necrosis in the entrance wounds was significantly higher than that in the exit wounds, the absence of epidermal/dermal necrosis in the exit wounds was significantly higher than that in the entrance wounds ($p<0.001$). The correlation of epidermal necrosis with the entrance and exit wounds is shown in Table 1. When the relationship between compact severe dermal necrosis and the entrance/exit wounds was examined (Figure 2), compact severe dermal necrosis was detected in 26 (41.9%) of the entrance wounds and in 2 (3.9%) of the exit wounds. The incidence of compact severe dermal necrosis in the entrance wounds was

significantly higher than that in the exit wounds (p<0.001). Moreover, the incidence of powder grains in the epidermis and powder grains and textile fibers in the dermis of the entrance wounds was significantly higher than that in the exit wounds (p<0.001, Table 2). When the relationship between dermal hemorrhage and the entrance/exit wounds was examined, focal dermal hemorrhage was detected in 41 (66.1%) of the entrance wounds and 44 (86.3%) of the exit wounds. Diffuse dermal hemorrhage was detected in 21 (33.9%) of the entrance wounds and 7 (13.7%) of the exit wounds. The incidence of diffuse dermal hemorrhage in the entrance wounds was significantly higher than that in the exit wounds (p=0.014). When the relationship between the adipose tissue in the dermis and the entrance/exit wounds was examined, adipose tissue was detected in the dermis in 5 (8.1%) of the entrance wounds and 11 (21.6%) of the exit wounds. The incidence of adipose tissue in the dermis of the exit wounds was significantly higher than that in the entrance wounds (p=0.04). Furthermore, in 3 (4.8%) of the entrance wounds, stratified squamous epithelium fragments were detected in the dermis. When the relationship between the muscle and bone tissue in the dermis and the entrance/exit wounds was examined, muscle and bone tissues were detected in the dermis in 3 (4.8%) of the entrance wounds and 18 (35.3%) of the exit wounds. The incidence of muscle and bone tissues in the dermis of the exit wounds was significantly

Table 1. Epidermal and dermal necrosis in the entry and exit wounds

	Entrance wound			Exit wound		
	Absent	Slight	Severe	Absent	Slight	Severe
Epidermal necrosis	0	19 (30.6%)	43 (69.4%)	20 (39.2%)	22 (43.1%)	9 (17.6%)
Dermal necrosis	0	4 (6.5%)	58 (93.5%)	23 (45.1%)	8 (15.7%)	20 (39.2%)

Table 2. Powder grains and textile fibers in the entrance and exit wounds

	Entrance wound		Exit wound	
	Epidermis	Dermis	Epidermis	Dermis
Powder grains	12 (19.8%)	26 (41.9%)	0	0
Textile fibers	3 (6.4%)	28 (59.6%)	0	5 (13.2%)

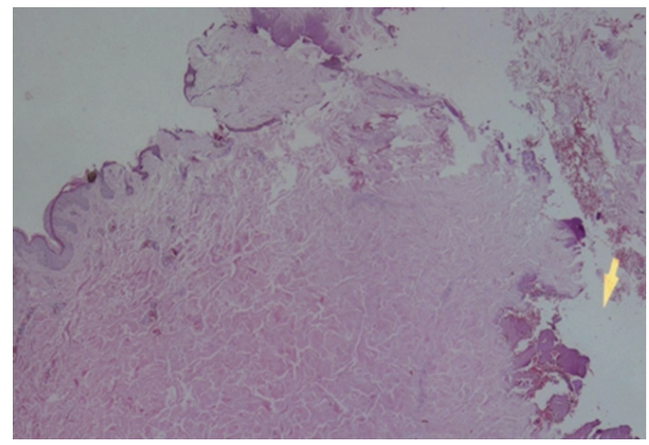

Figure 1. Magnification x4, significant fragmentation in the epidermis, fragmented severe necrotic tissue in the dermis (Entrance wounds).

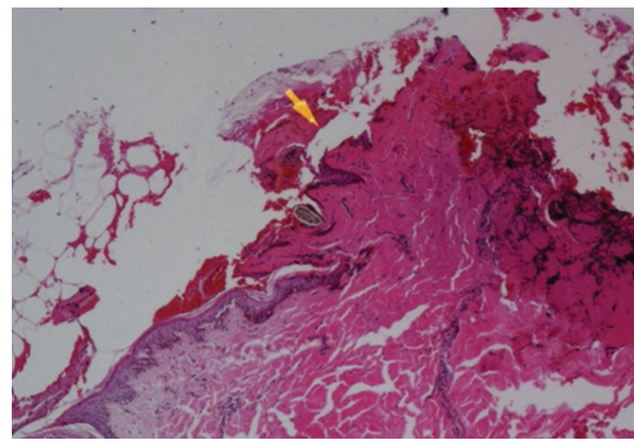

Figure 2. Magnification x10, severe epidermal necrosis, severe compact dermal necrosis, and the presence of black-pigmented powder particles in the dermis (Entrance wounds).

Table 3. Evaluation of the microscopic findings in the entrance wounds according to the range of fire.

		Contact range	Close range	Distant range	p value
Epidermal necrosis	Slight	4 (22.2%)	6 (66.7%)	8 (30.8%)	>0.05
	Severe	14 (77.8%)	3 (33.3%)	18 (69.2%)	
Dermal necrosis	Slight	0	1 (11.1%)	3 (11.5%)	>0.05
	Severe	18 (100%)	8 (88.9%)	23 (88.5%)	
Dermal hemorrhage	None or focal	10 (55.6%)	7 (77.8%)	19 (73.1%)	>0.05
	Diffuse	8 (44.4%)	2 (22.2%)	7 (26.9%)	
Subcutaneous hemorrhage	None or focal	3 (16.7%)	2 (22.2%)	8 (30.8%)	>0.05
	Diffuse	15 (83.3%)	7 (78.8%)	18 (69.2%)	
Powder grains in the epidermis	None	7 (38.9%)	8 (88.9%)	26 (100%)	<0.001
	Present	11 (61.1%)	1 (11.1%)	0	
Powder grains in the dermis	None	3 (16.7%)	2 (22.2%)	23 (88.5%)	<0.001
	Present	15 (83.3%)	7 (77.8%)	3 (11.5%)	
Necrotic tissue fragments covered by powder grains in the dermis	None	3 (16.7%)	2 (22.2%)	25 (96.2%)	<0.001
	Present	15 (83.3%)	7 (77.8%)	1 (3.8%)	
Textile fibers in the dermis	None	6 (85.7%)	1 (14.3%)	9 (37.5%)	0.019
	Present	1 (14.3%)	6 (85.7%)	15 (62.5%)	

higher than that in the entrance wounds (p<0.001).

In terms of the range of fire, there was no significant difference between epidermal necrosis, dermal necrosis, dermal hemorrhage, and subcutaneous hemorrhage in the examination of the entrance wounds. For the entrance wounds, the incidence of powder grains in the epidermis of the contact wounds was significantly higher than that of the close- and distant-range wounds (p<0.001). The incidence of powder particles in the dermis of the contact and close-range wounds was also significantly higher than that of the distant-range wounds (p<0.001). Similarly, the incidence of necrotic tissue fragments surrounded by powder particles in the dermis of the contact and close-range wounds was significantly higher than that of the distant-range wounds (p<0.001). For the entrance wounds in clothed areas, the incidence of textile fibers in the dermis of the close- and distant-range wounds was significantly higher than that of the contact wounds (p=0.019). The microscopic findings according to the range of fire are shown in Table 3.

Discussion

This is the first study conducted by the Institute of Forensic Medicine on the use of histopathology in distinguishing entry and exit of gunshot wounds and determining the shooting distance. In the study, histopathological parameters that can be used in the distinction of entry-exit wounds and determination of shooting distance were defined. Differentiating gunshot entrance and exit wounds and the range of fire are important in the resolution of forensic cases. Although related studies in the literature are limited, histopathological evaluations have been shown to be useful in determining entry/exit wounds and the range of fire [9-12]. In their study, Perez and Molina showed that microscopic examinations were not suitable for routine evaluations because some false positives were evident [7].

Baptista et al reported that necrosis (burns) was seen in the epidermis in all the entrance wounds in their study, but not in all the exit wounds [13]. However, in our study, epidermal necrosis was detected in some exit wounds. We believe that this is due to the greater number of wounds examined and their diversity. According to our results, the presence of severe epidermal necrosis is indicative that a wound is an entrance wound, while the presence of mild or no epidermal necrosis signifies that a wound is an exit wound.

In the study of Perez and Molina, soot and powder grains were detected in 60% of the entrance wounds in the tissue sections and 21% of the exit wounds [7]. In another study by Baptista et al, dermal powder grains were detected in 21.5% of the entrance wounds but not in the exit wounds [13]. The presence of powder particles in the entrance wounds in our study was consistent with the data in the literature, yet powder grains were not found in the epidermis or dermis of the exit wounds. Although the number of cases in our study was relatively high, we surmise that the absence of powder grains in the exit wounds may be related to the nature of the ammunition used. The supply of ammunition in Turkey is carried out by a single government agency, and ammunition other than full metal jacket (FMJ) bullets is very rare. The possible carrying effect of hollow-point (HP) ammunition may have created the difference in the study of Perez and Molina. However, Hlavaty et al8 stated that there was no difference between HP and FMJ rounds.

In the study of Baptista et al, adipose and muscle and bone tissue were not seen in any of the entrance wounds although adipose tissue was seen in 50% and muscle and bone tissue in 42.8% of the exit wounds [13]. In our study, we found a higher rate of adipose, muscle and bone tissues in the dermis of the exit wounds, which is consistent with the literature, and we posit that the presence of adipose, muscle, and bone tissues in the entrance wounds may be an artifact appearance due to the flow of blood resulting from the position of the body.

Powder particles might have been detected by histological examination in entrance wounds but not macroscopically [14]. In the study by Perez et al, while gunpowder particles and soot were not observed macroscopically, 9 of the 27 entrance wounds revealed powder grains microscopically [7]. Similarly, in our study, 3 of the 26 entrance wounds showed no macroscopic evidence of powder grains, but powder grains were present in the dermis. It is possible that these grains may have been transported by the bullets since bullet wipe can be seen in entrance wounds regardless of the range of fire [10].

There is no evidence in the literature that the presence of necrosis in entrance wounds can be used to determine the range of fire. In our study, no statistically significant correlation was found between mild and severe epidermal/dermal necrosis rates and the range of fire. Additionally, no statistically significant correlation was found between the skin and subcutaneous hemorrhage rates and the range of fire.

Necrotic tissue fragments surrounded by black-pigmented particles were observed in 83.3% of the contact wounds, 77.8% of the close-range wounds, and 3.8% of the distant-range wounds, so we determined that the presence of GSR in the skin decreased as the range of fire increased. These findings are consistent with those in the literature.

In our study, the incidence of powder grains in the epidermis of the contact wounds was significantly higher than that in the epidermis of the close- and distant-range wounds, while their incidence in the dermis was significantly higher for the contact and close-range wounds than for the distant-range wounds. Hlavaty et al stated that there is no correlation between macroscopic and microscopic examinations in determining range of fire [15]. The most important factors determining the behavior of powder grains were not discerned in either study. The physical form of gunpowder grains affects the distance they travel, so the available data were insufficient to reach a scientific conclusion [10].

The use of standard histological examination methods in gunshot wound cases depends on the circumstances. Microscopic examinations are recommended in selected cases; however, there are diverse types of bullets and gunpowder. Such a large number of variables constitutes an obstacle to the establishment of diagnostic criteria for microscopic examination. Further studies in this regard are therefore warranted.

Conclusion

In conclusion, in cases of gunshot wounds, histopathological evaluation, in addition to other methods, is supportive in confirming the diagnosis. At the same time, we think that in cases where the integrity of the corpse is disrupted,

clothing is absent, intervention has been carried out and/or wound characteristics cannot be evaluated macroscopically, histopathological examination is a method that should be used and will guide us in determining whether the wound is a gunshot wound or not, as well as in distinguishing between entry and exit wounds and estimating the distance.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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